EXPERIMENTAL BIOLOGY

Transplantation of Cultured Human Neural Progenitor Cells into Rat Brain: Migration and Differentiation

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We studied the fate *in vitro* cultured human stem/progenitor cells after transplantation into rat brain. The cells from human fetuses at 8-12 weeks' gestation were cultured *in vitro* for 14 days and transplanted into the brain of 10-day-old and adult rats. Microscopic examination showed that human stem/progenitor cells migrated into various regions of rat brain. Immunohistochemical assay demonstrated that some cells differentiated into astrocytes and neurons, while others retained the embryonic phenotype.

Key Words: *human neural stem/progenitor cells; cultured cells; transplantation; differentiation*

The interest in biology of neural stem cells is related not only to fundamental problems of cell differentiation [3,9,10], but also to the possibility of using these cells in clinical practice for correction of human brain diseases [1,4-6,13,15].

Recent studies showed that stem cells playing an important role in embryogenesis and early postnatal ontogeny are present in some brain regions in adult mammals and humans [2,8,12]. The mechanisms regulating functions of stem cells in the embryonic and differentiated brain are poorly understood. The main directions of recent studies were isolation, division, and maintenance of these cells in tissue cultures and evaluation of their *in vitro* differentiation capacities after transplantation into the brain.

Here we studied the development and differentiation of stem/progenitor cells (SPC) from human fetal brain cultured *in vitro* and transplanted into rat brain.

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MATERIALS AND METHODS

The brain obtained from human fetuses at 8-12 weeks' gestation was suspended and the cells were cultured in NPBM medium (Clonetics) supplemented with standard growth factors (fibroblast growth factor, epidermal growth factor, and type 1 neuronal survival factor), gentamicin, and amphotericin B (NPMM, Clonetics).

For visualization of human SPC in rat brain the cells were stained with DNA-specific fluorochrome bisbenzimide (Hoechst 33342, Serva) before transplantation.

Wistar rats (n=50) served as recipients. The cells were stereotactically transplanted into the lateral cerebral ventricle of 10-day-old rat pups (n=8) and 2-month-old adult rats (n=16) or into the cerebellum of 26 adult rats. Adult animals were intraperitoneally narcotized with 300 mg/kg chloral hydrate. Rat pups were narcotized by placing on ice. The suspension of human SPC in a concentration of 4×10^7 cells/ml (4 μl, total cell count 1.6×10^5) was introduced into the brain. Immunosuppression was not performed.

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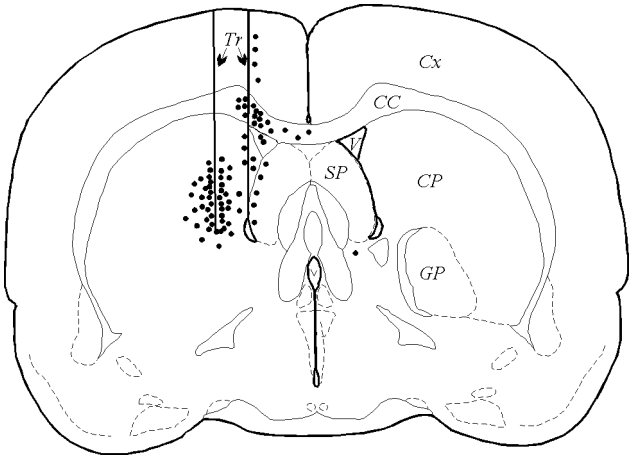

Fig. 1. Transplantation of cultured human stem/progenitor cells into rat brain. The scheme represents tracks of inoculation and migration form the site of transplantation in the recipient brain: corpus callosum (*CC*), brain cortex (*Cx*), caudate nucleus-putamen (*CP*), septum (*SP*), and globus pallidus (*GP*). Here and in Fig. 2: *Tr*: track of inoculation; *V*: cerebral ventricles.

The animals were transcardially perfused with 4% paraformaldehyde in 1 M phosphate buffer (pH 7.3) 10 and 20 days after transplantation. The brain was removed and placed into a fixative (for 24 h) and 30% sucrose. Sections (20-40 μ) were prepared on a freezing microtome, examined under a luminescence microscope, and stained with cresyl violet or immunohistochemically.

Brain sections and cultured cells fixed by the same method were assayed immunohistochemically using primary antibodies to nestin (Biogenesis, 1:20), gliofibrillar acid protein (GFAP, DAKO, 1:250), β-tubulin (ICN, 1:100), vimentin (NeoMarkers, 1:100), and calbindin (Sigma, 1:1000). The cells and cryostat sections were treated with 0.3% Triton X-100 and incubated overnight with primary antibodies. After washout the material was treated with biotinylated secondary antibodies (Vector Laboratories) and streptavidin labeled with fluorescent probe DiI (Molecular Probes). The samples were washed 3 times and clarified with 50% glycerol in phosphate buffered saline.

RESULTS

In a selective medium the primary suspensions of human SPC formed tissue fragments, aggregates and neurospheres, which progressively increased in size during culturing. These tissue fragments are below named cell clusters (as in previous reports [10,14]). After 14-day culturing some cells were transplanted into rat brain, while others were transferred into a serum-containing medium, fixed on the next day, and studied immunocytochemically.

Immunocytochemical assay showed that multipotent cells, which formed cell clusters in culture, underwent differentiation by the neural or glial type. At this developmental stage clusters included true stem (nestin-positive), progenitor (vimentin-positive), and differentiating cells expressing GFAP and β-tubulin. Therefore, these clusters were heterogeneous by their cell composition, which confirmed the results obtained by M. K. Carpenter *et al*. [7].

Microscopic examination revealed transplants of cultured human SPC in the brain of recipient animals 10 and 20 days after transplantation (40 and 10 rats, respectively). Groups of intensively fluorescing transplanted cells were localized in the brain cortex, white matter, lateral ventricle, caudate nucleus, and in all cerebellar layers (inoculation track). Transplanted cells were surrounded by individual macrophages and small necrotic zones, but no pronounced immune reactions and tumors were seen.

The density of implanted cells decreased from the site of injection to the periphery. In dense regions of transplants we revealed cells undergoing mitotic division, which indicated that they retained proliferative activity in a new microenvironment.

Transplanted cells were divided into 2 populations: cells with small, elongated, and intensively fluorescing nuclei (group 1) and large cells with light and oval nuclei (group 2). Group 1 cells intensively migrated into the brain parenchyma, while group 2 cells were characterized by less pronounced migration.

Cells transplanted into the lateral cerebral ventricle in 8 rat pups and 16 adult rats were found in the neocortex, white matter, and caudate nucleus and near or inside the lateral ventricle (Fig. 1). These cells migrated along the ventricular epithelial layer. Small cells were localized in the lateral ventricular wall at a distance of 1.5-2.0 mm, while large cells migrated by 200-300 μ from the site of injection. The cells most intensively migrated in the white matter, where they were localized at a distance of 2-3 mm from the site

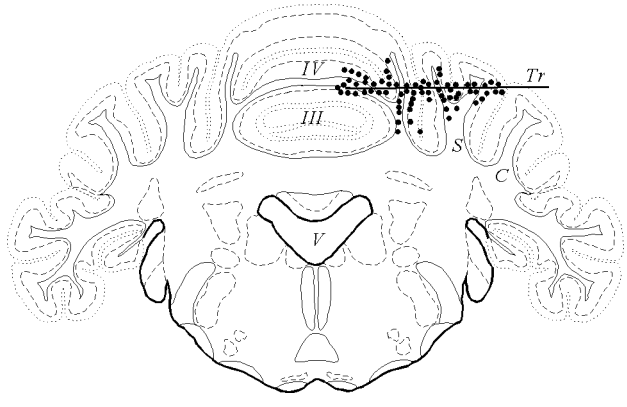

Fig. 2. Transplantation of cultured human stem/progenitor cells into rat cerebellum. The scheme represents zones of migration of transplanted cells into cerebellar tissue. *III*, *IV*, *S*, and *C*: cerebellar lobes.

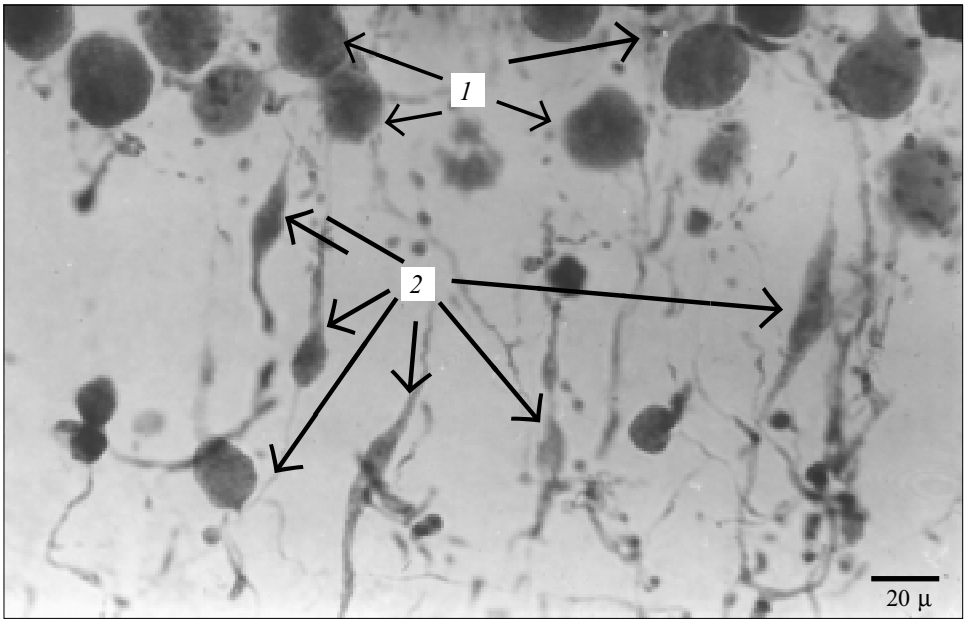

Fig. 3. Purkinje cells (*1*) and transplanted human stem/progenitor cells (*2*) in rat cerebellum. Staining with anticalbindin antibodies(×400).

of injection or reached the opposite hemisphere. In the cortex cell migration was less pronounced.

Group 2 cells migrated less intensively. They were densely positioned in the central zone of the transplants. However, individual cells migrated into the brain parenchyma by 150-300 μ from the site of injection (marginal zone of the lateral ventricle, caudate nucleus, and brain cortex).

In the brain of recipients we found small neuronal satellite cells and cells adjacent to blood vessels. Immunohistochemical staining for GFAP showed that these cells were astrocytes. Nuclei of individual small cells were stained with bisbenzimide and surrounded by dense layers of GFAP-positive fibers, which confirmed results of R. Friker *et al.* [10].

Immunohistochemical staining for antibodies to vimentin revealed the presence of cells expressing this protein, which is typical of immature non-committed cells. Therefore, the transplant contained embryonic cells not undergoing differentiation.

Staining with antibodies to calbindin showed that multipotent stem cell underwent differentiation by the neural type. Double staining revealed antibodies bound to individual neural cells, whose nuclei contained bisbenzimide.

No differences in morphological signs, state, and differentiation of cells transplanted into the brain in newborn and adult rats were found. These cells demonstrated similar migration and differentiation capacities. Moreover, we found no immune and glial reactions in recipient brain.

Human cultured cells transplanted into the cerebellum were visualized in all animals (n=26). Cultured cells migrated into the molecular, granular, and Purkinje cell layers and the white matter (Fig. 2). These cells most intensively migrated into fibrous cerebellar structures (similar to the forebrain).

Transplanted cells labeled with bisbenzimide were detected near Purkinje cells in recipients. Immunohistochemical staining with antibodies to calbindin showed that some labeled cells expressed this marker (Fig. 3). The embryonic phenotype of cells and expression of calbindin suggested that some human SPC undergo differentiation into Purkinje cells, which constitute the only population of calbindin-positive cells in the cerebellum (Fig. 3).

Thus, cultured human neural SPC transplanted into rat forebrain and cerebellum survive not less than for 20 days, retain the ability to migrate and undergo division, and differentiate by the glial or neuronal type.

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